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ABSTRACT

This study investigates the relevance of several soil chemical extractions ($CaCl_2$, acetic acid, citric acid and a four-step sequential procedure) and of predicted free metal ion activities in the soil solution (according to equations using total concentrations and pH) to characterize the transfer of trace metals (Cd, Pb and Zn) from soil to snail soft tissues over a large smelter-impacted area (Metaleurop Nord, Nord-Pas-de-Calais, France). The study was firstly performed on 6 wild snail species together and then, specifically, on *Cepaea* sp and *Oxychilus draparnaudi*, which were well distributed over the study area and have contrasted diet (*Cepaea* snails are herbivorous while *O. draparnaudi* is carnivorous). When the 6 species were considered together, the accumulation depended most on the species, total or extractable metal concentrations in soils, or predicted free ion activities, accounting for less than 7% of the variation of metal concentrations in snail tissues. Species-specific analyses showed that extractable concentrations explained around 25% of the variation of metal concentrations in *O. draparnaudi* while it was up to 8% for *Cepaea* snails. When using total soil concentrations and soil properties as explanatory variables, models were generally slightly better, especially in species-specific analyses, explaining up to 42% of the variance. These results show that the soil extraction procedures used in this study, and predicted free ion activities of soil pore water, could not accurately estimate metal transfer from the environment to snails, probably because they do not take into account other sources of exposure than soil, and thus could not be used in risk assessment. Insight has to be gained into the determination on food web structure and composition and subsequent contaminant transfers, in order to improve risk assessment for such organisms.

Keywords

Biological monitoring, bioavailability, snails, toxic metals.

1. Introduction

Due to historical and modern industrial activities, soils can be severely contaminated with metals (e.g. cadmium, Cd; lead, Pb; and zinc, Zn) and the release of such contaminants in natural systems can consequently pose environmental and human health risks. In the case of areas affected by metallurgical industries, the original load is primarily sourced from smelter fallout and dusts from spoil heaps that have been transported across natural systems. Exposure of organisms to trace metals depends on many non-biotic factors (including, for instance, the spatial distribution of metals in soils, landscape or habitat features, season etc) as well as the biological characteristics of the organism itself like its diet and, more generally, its life history traits (Suter II, 1993). In line with that, Landrum et al. (1994) developed the concept of environmental bioavailability as the fraction of the environmental availability (modulated by physicochemically-driven processes) that is available over the exposure period for uptake (physiologically-driven processes) by a given species (Peijnenburg and Jager, 2003).

In the framework of ecotoxicology and ecological risk assessment, terrestrial invertebrates have been extensively studied as biomonitors of environmental contamination by metals. The relationships between concentrations of metals in snails and in soils have been well studied in controlled conditions as well as on the field. Those studies generally have shown that concentrations in snail tissues are related to the contamination of their environment (Berger and Dallinger, 1993). The biota-to-soil concentrations ratios (BSAFs) that can be derived from these studies may be useful in ecological risk assessment (Veltman et al., 2008). However, relationships between total metal concentrations in soil and concentrations in snail soft tissues are usually relatively low, hampering accurately predicting concentrations in organisms from total soil concentrations. For instance, coefficient of determination (R^2) of the relationship between *Cepaea nemoralis* snail and soil concentrations were of 0.33, 0.17 and 0.15 for Cd, Pb and Zn, respectively (Notten et al., 2005). Such low relationships may be due to the fact that total metal concentrations are usually considered not to be the best estimate of bioavailability for both plants and organisms (Harmsen, 2007).

In order to overcome this limitation, a variety of chemical methods has been developed to estimate the available fraction of metals in soils, and then to correlate these available concentrations with metal levels in tissues or with effects observed in organisms. Among these methods, single or sequential extraction techniques have given satisfactory results for certain pollutants, certain soils and/or certain organisms (see, for instance, Harmsen, 2007; Meers et al., 2007; Hass and Fine, 2010), even if it is clear that no universal extractant exists. Free metal ion activity in the soil solution has also been suggested as a pertinent estimate of metal bioavailability and toxicity in aquatic organisms and higher plants (Smolders et al., 2009) and may therefore be hypothesised as a good predictor of metal concentrations in soil organisms (Sauvé et al., 2000). Though, studies examining relationships between metal concentrations in soil organisms and free ion activities in pore water or extractable metal concentrations in soil are scarce.

Within this context, we hypothesized that chemically extractable metal concentrations in soils, or predicted free ion activity, would give better relationships with metal concentrations in snail tissues, than total metal concentrations in soils. We thus aimed at comparing the relationships between total or extractable (using three selective extractions and a four-step sequential procedure) soil concentrations or predicted free ion activities and metal concentrations in snail soft tissues. As an alternative to the use of extractable concentrations, we also modelled metal concentrations in snail tissues using total metal concentrations in soils and soil properties.

2. Materials and methods

2.1. Study site and sampling strategy

This study was carried out in the surroundings of the former “Metaleurop-Nord” smelter in Northern France (Noyelles-Godault, Nord-Pas-de-Calais, 50°25’42 N 3°00’55 E). This area is considered as highly polluted with Cd, Pb and Zn for both levels of contamination and surfaces of concern (Sterckeman et al., 2002b; Douay et al., 2008; Douay et al., 2009; Fritsch et al., 2010). A 40 km^2 (8 x 5 km) study area, centred on the former smelter, was defined. This area was split into 160 squares (500 m-sided), which constituted our sampling units. Because of logistical constraints, the present study was performed on 30 squares located along a soil pollution gradient, chosen among the 160. In each of the 30 squares, one to 10 composite soil samples (constituted each by 15 randomly placed sub-samples in a homogeneous patch) were taken in woody patches (woodlots, hedgerows, tree plantations, etc) during autumn 2006. Soil sampling was carried out on the first 25 cm and the litter layer (OL layer constituted by the accumulation of no- or few decomposed leaves and woody fragments on the soil surface) was removed. However, the humus layer (OF layer constituted of fragmented residues) was sampled with the top mineral soil material, in accordance with the most frequently recommended protocol in Europe. Detailed data about soil sampling, soil physico-chemical characteristics and contamination are given in Douay et al (Douay et al., 2009) and Fritsch et al (Fritsch et al., 2010). Snails were hand-searched during autumn 2006 in patches where soils were sampled. Snails were sampled in the morning, stored in plastic bags without food and frozen at night. This allowed a depuration period of around 8 hours.

2.2. Determination of snail species and age and preparation of snail tissues

Species were determined according to morphometric criteria (Kerney and Cameron, 2006). Snails belonging to the *Cepaea* genus were classified according to two classes of relative age (adults or juveniles), according to the presence or the absence, respectively, of a clear white or brown-black lip at the mouth of their shell (Williamson, 1979). The presence of this lip indicates that the snail has attained adulthood. In these *Cepaea* snails, the determination at a species level is only possible on adults. In the case of non reproductive juveniles, the snails were called "*Cepaea* juveniles". Class age was not determined for the other snail species due to the absence of published ageing method and/or to insufficient sample size to take age into account in statistical treatments.

The soft body of the snails was separated from the shell and dried in an oven (60°C) to constant dry weight before metals analysis. Snails were generally analysed individually, but when their dry mass was lower than 0.1 g, two to 10 individuals (depending on the mass of the individuals) were pooled to obtain a sufficient biomass for metals analysis. For both individual and pooled samples, snail metal concentrations were normalized to soft tissues weight.

2.3. Analyses of metals concentrations in soils and animals

Soils were analyzed for total and extractable metal concentrations and for soil properties. The protocols are detailed in Douay et al. (2009). Briefly, samples were dried, disaggregated and homogenized before sieving to 250 µm. The following soil characteristics were measured: granulometry, pH (water suspension), organic carbon content (OC), organic matter (OM), total carbonate content ($CaCO_3$) and cation exchange capacity (CEC). Total Cd and Pb concentrations were measured by inductively-coupled argon plasma mass spectrophotometry (ICP-MS) and Zn concentrations by inductively-coupled argon plasma atomic emission spectrophotometry (ICP-AES) after a total digestion with a mixture of hydrofluoric (HF) and perchloric ($HClO_4$) acids. Soil properties as well as metals concentrations in soils were analyzed by the *Laboratoire d'Analyse des Sols* of the *Institut National de la Recherche Agronomique* (INRA), of Arras (France), which is accredited by the *French Accreditation Committee* (COFRAC, n°1-1380) for the analytical quality for soil characteristics and metals concentration measurements.

Three selective extractions and one sequential four-step extraction were chosen. Calcium chloride ($CaCl_2$, 0.01M) was chosen to estimate cation exchange reactions and because it is considered as relevant for the assessment of the bioavailable fraction of Cd, Pb and Zn to plants and soil invertebrates (Harmsen, 2007; ISO, 2008). Acetic acid ($C_2H_4O_2$, 0.11M) and citric acid ($C_6H_8O_7$, 0.11M) are two low molecular weight organic acids (LMWOA) mostly present in the rhizosphere of many plants and used to simulate complexing behaviour of root exudates (Meers et al., 2007). These two LMWOA increase the overall metal solubility by dissolution of hydroxides and carbonates and by acting as complexing agents. Acetic acid extractable fraction has been used as an estimate of the metal bioavailability to soil animals (Morgan and Morgan, 1999; Lukkari et al., 2004). Citric acid has been used to simulate the physicochemical characteristics of root environment of the plant (Ahumada et al., 2004). A three-stage sequential extraction procedure was also performed, allowing the extraction of four operationally defined fractions: acid-soluble extracted by 0.11M acetic acid (exchangeable and carbonate bound), reducible extracted by 0.5 M of hydroxylammonium chloride at pH 2 (Fe- and Mn-oxide bound), oxidizable extracted by 8.8 M of H_2O_2 followed by 1 M of ammonium acetate at pH 2 (organic matter and sulfide bound) and residual extracted by hydrofluoric (48 %) and perchloric (70 %) acids (included within silicate matrix). The extractable concentrations of those four fractions will be called a, b, d and r, respectively, later in this article. Extractable metal concentrations were quantified using atomic absorption spectrometry (AAS, AA-6800, Shimadzu) by the *Laboratoire Sols et Environnement* of the *Institut Supérieur d'Agriculture* (ISA), Lille, France. Three independent replicates were performed for each sample and blanks were measured in parallel for each set of analyses, measurements of certified reference materials (i.e. BCR-701 and BCR-483) was were used to ensure the quality of the results obtained. For all metals average recoveries of the CRMs were at XX% ± 10%.

Finally, predicted free ion activities in the soil solution (pPb^{2+}, pCd^{2+}, pZn^{2+}) were calculated from pH and total metal concentrations using the equations proposed by (Sauvé et al., 1997; Sauvé et al., 2000; Stephan et al., 2008), respectively. As calculated by those equations, the activity of metal ions corresponds to -log(free ion concentration in M).

Metal concentrations in snail soft bodies were measured by furnace (Cd, Pb) or flame (Zn) atomic absorption spectrometry (VARIAN 220Z and 220FS, respectively) at the Chrono-Environment Department of the University of Franche-Comté, Besançon, France. Digestion of samples was performed using dissolution in nitric acid (HNO_3, 65%, Carlo-Erba analytical quality) in a dry oven (65°C) during 72h. After digestion, samples were diluted adding ultra-pure water (18.2 $M\Omega.cm^{-2}$). Blanks (acid + ultra-pure water) and Certified Reference Materials (CRMs, TORT-2 and DOLT-3, National Research Council, Canada) were prepared and analyzed using the same methods.

Average recoveries of the CRMs were at 95% ± 10% (n = 38) for Cd, 101% ± 17% (n = 42) for Pb, 80% ± 2% (n = 22) for Zn. Detection limits in snail soft bodies were 0.14, 0.20 and 4.4 $\mu g.g^{-1}$ for Cd, Pb and Zn, respectively. For both soils and animals, metal concentrations were expressed as micrograms per grams dry weight ($\mu g.g^{-1}$ dw).

2.4. Statistical analyses

For each sampled individual, the following data were available: snail species, metal concentrations in soft tissues, and age for *Cepaea* snails. Environmental data available were total and extractable metal concentrations and soil properties (clay, silt, sand, pH, $CaCO_3$, CEC, OC and OM) in the soil of the patch where the individual was caught. The statistical distribution of the data was checked with the test of Shapiro. Because metal concentrations in soils and animals were skewed, variables were $\log_{10}(x + 1)$ transformed for statistical analyses. These transformations allowed matching the assumptions of normality and linearity which are required to perform General Linear Models (GLM).

First, principal component analyses were performed on soil properties to identify redundant variables. According to those analyses, pH, OM, CEC, clay and silt were retained for further analyses. Simple linear regressions were also performed to explore relationships between snail and soil concentrations, and between biotic (species or age) and abiotic variables (soil properties or metal concentrations).

Second, relationships between metal concentrations in snail tissues and in soils (total or extractable concentrations, or predicted free ion activities in the soil pore water) were determined. General Linear models (GLM) (Grafen and Hails, 2002) were performed using metal concentration in snail tissues as dependant variable and species and metal concentrations in soils as explanatory variables. Specific analyses were then made for *O. draparnaudi and Cepaea sp.* because of their relative abundance and their homogeneous spatial distribution over the study area. Moreover, these two species have clearly distinct diet, *O. draparnaudi* being carnivorous while *Cepaea* snails are herbivorous (Kerney and Cameron, 2006). Concentrations of metals in *O. draparnaudi* were modeled using soil concentrations as only explanatory variable. For *Cepaea* snails, preliminary analyses (linear models) showed no difference of concentration between adult *C. nemoralis* and *C. hortensis*, whatever the metal. Metal concentrations in *Cepaea sp* individuals were thus modeled using age and soil concentrations as explanatory variables, without further considering the species.

Third, GLM involving concentrations in snails tissues as a dependent variable and total metal concentrations in soils and soil properties as explanatory variables were performed. Preliminary monovariate analyses having shown that biotic variables (e.g. species when analyses were performed on the six snail species and age for *Cepaea* snails) were of primary importance in explaining metal concentrations in tissues, they were placed first in the models. Then, to assess the influence of soil properties conditionally to total soil concentrations, this last variable was placed in second in the models. These two variables in the model being fixed (biotic variables and total concentration), soil properties were added. Precisely, the order of soil properties in the models was chosen according to preliminary monovariate analyses linking concentrations in snails to soil properties. All soil variables were placed in the model in the decreasing order of their coefficient of determination (R^2)

obtained in monovariate analyses. The complete model was then analysed by ANOVA and non-significant variables were excluded.

Post-hoc multiple comparisons were performed using Tukey's Honest Significant Difference test ($p < 0.05$) to test significant differences of metal concentrations between species. Biota-to-Soil Accumulation Factors (BSAFs) were calculated as the ratio of metal concentration in the soft tissues on total metal concentration in the soil. The relationship between BSAF and total soil concentration (conditionally to species) was assessed using a linear models.

When models had two variables, interactions between the variables were studied when relevant. For all tests, significance was chosen at $p<0.05$. All statistical analyses were performed using the software R 2.10.1 with *pgirmess* and *ade4* packages (RDevelopmentCoreTeam, 2010).

3. Results

3.1. Snail species, metal concentrations in soils and in snail and BSAF

In the present paper, analyses were conducted on six out of the 19 species sampled. These six species were selected because they were relatively abundant and well spatially distributed over the study area. A total of 264 snail samples were found in 48 habitats within the 30 squares where they were sampled. The 264 snails include 20 copse snails (*Arianta arbustorum*), 47 white-lipped banded snails (*Cepaea hortensis*), 54 brown-lipped grove snails (*Cepaea nemoralis*) and 30 juvenile *Cepaea sp* snails, 19 garden snails (*Cantareus aspersus*, ex-*Helix aspersa*), 78 Draparnaudi's glass snails (*Oxychilus draparnaudi*) and 16 strawberry snails (*Trichia striolata*).

Soils from the study site were heavily polluted with metals (Table 1). Median total concentrations of Cd, Pb and Zn were much higher than the background values of woody topsoils of the region which are 0.2, 53.3 and 53.4 µg g^{-1}, respectively (Sterckeman et al., 2002a), except six topsoils that exhibited background concentrations. Soil properties varied greatly according to the different sampling sites (Table 1). Topsoil texture was mainly loamy to silty-loamy. All other soil properties exhibited large variations, up to one order of magnitude for organic matter content, showing a great variability of types of soils within the study area.

Metal concentrations in snail soft bodies were characterized by distinct patterns, which varied between species and as a function of the element considered (Table 1, Figure1). Median Cd concentrations in snails (45.1 µg.g^{-1}) were higher than those measured in soils ($p < 0.001$). Only *Arianta arbustorum* and juvenile *Cepaea sp* snails exhibited significant lower Cd concentration compared to other species (Figure 1). Inversely, Pb concentrations in snail tissues (median Pb concentration of 33.2 µg.g^{-1}) were lower than those measured in soils ($p < 0.001$). No significant intra-species differences were detected for Pb concentrations. Finally, Zn concentrations in snails were of the same order of magnitude than those found in soils ($p = 0.58$). Higher Pb concentrations were measured for *Oxychilus draparnaudi* and *Trichia striolata* compared to other species (Figure 1).

Cd biota-to-soil accumulation factors (BSAF) were consistently higher than 1 for all species (Figure 2). The transfer of this metal was the lowest for *A. arbustorum* (median BSAF of 1.2) and juvenile Cepaea snails (median BSAF of 1.7), and the highest for *T. striolata* (median BSAF of 50.6) (data not shown). The four other species exhibited similar intermediate median BSAFs ranging from 3.5 to 7.4. For Pb, median values of BSAF between species ranged from 0.031 to 0.13 and did not show inter-species differences, except for *T. striolata*, for which BSAFs reached 0.53. The inter-species pattern was similar for Zn, with all species exhibiting median BSAFs ranging from 0.49 to 1.23, while it was 15.0 for *T. striolata*.

When considering all species together, the relationship of BSAF to exposure was significant and negative for all the metals considered (Figure 2, left). Comparing the carnivorous *O. draparnaudi* to the herbivorous *Cepaea sp.* (Figure 2, right), the decrease of BSAF values for Cd and Pb along the pollution gradient were significantly higher for *Cepaea* snails (slopes = -0.93 and -0.12, respectively) than for *O. draparnaudi* (slopes = -0.41 and -0.05, respectively). For Zn, BASF decreased similarly for *Oxychilus* (slope = -0.35) and *Cepaea* snails (slope = -0.37).

3.2 Influence of total or extractable concentrations, and of free metal ion activities in the soil solution on concentrations in snail tissues

Whatever the extraction, Cd concentrations in the 6 species of snails were poorly (at best 6.8%) explained by total or extractable soil concentrations (Table 2). Three of the four steps of the sequential procedure even led to non significant relationships. No relationship (except for Pb_b concentrations) was evidenced for Pb. Zn concentrations in snail tissues were very poorly explained by soil concentrations. Whatever the metal, the influence of the species was always higher than that of soil concentrations (total or extractable) in explaining metal concentrations in snail soft tissues. Species and Pb_b extracted concentrations accounted for 11% of the variation of Pb concentrations in snail tissues. Depending on the considered extraction, species and metal concentrations explained 18.3 to 21.7% of the variance for Cd, and from 35.4 to 38.5% for Zn. For both metals, however, some extractable concentrations were not significantly related to snail concentrations. The use of predicted free activities led to similar low correlations for each metal (Table 2).

Considering all extractions together, the relationships between concentrations in *O. draparnaudi* snails and soil concentrations were much better than when all species were considered together and also better than for *Cepaea* snails (Table 2). Metal concentrations in *O. draparnaudi* tissues were never significantly correlated with free ion activities in the soil solution. Cadmium levels in *O. draparnaudi* increased significantly with total as well as extractable (all extractants) Cd concentrations in soils (Table 2). The various extractions explained from 5.5 (Cd_d) to 24.3% (acetic acid) of the variations of Cd concentrations in snails. Total as well as Pb_d concentrations were poorly related to Pb concentrations in snail tissues. Three other extractable concentrations (citric and acetic acids, and Pb_b) explained from 19.1 to 25.3% of the variations of Pb concentrations. $CaCl_2$-extractable concentrations were the only non-significantly related concentrations for Zn. Zinc concentrations in snail tissues were best related (25.2% of variance explained) to Zn_b concentrations. Total concentrations and the other extractants explained 13.7 and from 6.4 to 10.9% of the variations of Zn concentrations in snails, respectively.

For *Cepaea* snails, two extractable Cd concentrations ($CaCl_2$ and Cd_b) and pCd^{2+} were significantly related to concentrations in soft tissues. For Pb, only acetic acid extractable concentrations and pPb^{2+} were significantly related to *Cepaea* tissue concentrations, the relationship being negative for acetic acid. $CaCl_2$-extractable and Zn_r concentrations, and pZn^{2+} were significantly correlated with Zn concentrations in *Cepaea* tissues. Inversely to *O. draparnaudi*, predicted free ion activities in the soil pore water were significantly correlated to concentrations in *Cepaea* for all metals. Although Cd concentrations in *Cepaea* tissues were age-dependant, this was not the case for Pb and Zn (Table 2).

Generally speaking, for all metals, extractable concentrations were weakly correlated with snail concentrations when the six species were considered together. A specific analysis shows that few extractable concentrations were significantly although weakly related to *Cepaea* tissues. In *O. draparnaudi*, extractable concentrations better correlated with concentrations in snail tissues but the best extractant explained 24.3, 25.3 and 25.2% of the variance for Cd, Pb and Zn, respectively.

3.3. Influence of soil properties on concentrations in snail tissues

Models using soil properties (conditionally to species or age and total soil concentrations) were not more efficient (adjusted R^2 of the models of 13.4, 23.0 and 41.6% for Pb, Cd and Zn, respectively) than extraction-based models to explain concentrations in soft tissues for the six species considered together (Table 3). The species still was the most important explaining variable. Considering the influence of total concentrations and soil properties separately (partial R^2), conditionally to species, total soil concentration was shown to be weakly related to concentrations in snails and was even non significant for Pb. No clear trend concerning the influence of soil properties could be evidenced: one to three soil properties appeared to be significant depending on the metal of concern and, whatever the metal, explained no more than 4.6% of the variance of snail concentrations.

As observed for extraction-based models, Pb and Zn concentrations in snail soft tissues were better explained for *O. draparnaudi* than for *Cepaea sp* (Table 3). For Cd, adjusted R^2 models were similar for both species with 30.0% for *O. draparnaudi* and 29.0% for *Cepaea*.

Metal concentrations in *O. draparnaudi* were positively related with total metal concentrations in soils and negatively with organic matter contents. For Cd, the influence of soil concentration was higher than that of organic matter whereas it was the contrary for Pb and Zn.

For *Cepaea*, models involved more soil variables than for *O. draparnaudi*. As for extraction-based models, the age was an important variable to explain Cd concentrations in soft tissues. Its importance was lower for Pb and not significant for Zn. Total metal concentrations in soils were never significant for this species. Silt and pH influenced negatively snail concentrations for all metals. Organic matter was negatively correlated with snail concentrations for Cd, whereas it was positively correlated for Pb and Zn.

4. Discussion

4.1. BSAFs are influenced by both the species and the total soil concentration

For most species, median values of BSAF were around 5 for Cd, which is in accordance with Martin and Coughtrey who showed Cd bioaccumulation factors of 1.37–7.57 in various species of snails (Martin and Coughtrey, 1982). Few inter-species differences were noted for Pb, median BSAFs being lower than 0.5 for all species, indicating either an important excretion and/or a low assimilation of this metal. Except for *Trichia striolata*, median BSAFs for Zn values generally ranged from 0.5 to 1. Our data showed a negative relationship between BSAFs and total soil concentrations for all investigated metals. The highest BCF values for all metals were at low exposure concentrations, while the lowest BCF values were at elevated metal exposure levels. These results are consistent with those of Spurgeon and Hopkin (Spurgeon and Hopkin, 1996) and Veltman et al (Veltman et al., 2008), who found inverse relationships between BASFs in animals from all trophic levels and metal concentrations in soils. Similarly, this trend has also been described in aquatic environments, with various aquatic species (McGeer et al., 2003). The fact that the BSAF decreases linearly with increasing soil concentration, almost with a slope of 1.0, indicates a significant degree of control over metal accumulation, probably regulation. A constant BSAF would in fact suggest the contrary, namely that metals level in the snails does increase with increasing soil concentrations. Consequently, our results suggest that the degree of control over metal accumulation was stronger for Cd than for Zn. The relatively gentle slopes observed for Pb indicate that BSAF appear to be independent of the soil concentration. Moreover, slope differences observed for the carnivorous *O. draparnaudi* with the herbivorous *Cepaea sp.* suggest the influence of species for metal accumulation and regulation.

The physiological basis for the inverse relationship of BSAF to metal exposure concentration arises from metal uptake and control mechanisms (McGeer et al., 2003). Lock and Janssen (2001) concluded that uptake rate constants for cadmium decrease with increasing metal concentration, based on experimental results on two terrestrial oligochaetes, *Eisena fetida* and *Enchytraeus albidus*. At low environmental Zn levels, snails are able to sequester and retain Zn in tissues for essential functions. When Zn exposure levels are elevated, snails are able to control bioaccumulation. Despite it is known that *Cantareus aspersus* snails are able to regulate Pb concentrations more closely than other metals (Beeby and Richmond, 2010), our results indicate no-regulation controls on Pb bioaccumulation. These authors suggest that BSAF are unreliable indicators of an evolved regulatory mechanism for Pb.

4.2. Total and extractable metal concentrations in soils are poor predictors of metal concentrations in snail tissues

While selective and sequential extractions are frequently considered as a promising tool to measure environmental availability of trace metals in soils (Harmsen, 2007; ISO, 2008), relatively few data are available on relationships between extractable concentrations from field soils and concentrations in tissues of wild animals. Our data show that extractable concentrations from three selective and one sequential four-step extractions, as well as predicted free ions activities in the soil solution, explain up to 42% of the variations of the concentrations of Cd, Pb and Zn in the tissues of snails sampled in the surroundings of a former Pb-smelter and belonging to six species.

Among the data relating metal concentrations in wild animals with various soil extracts, earthworms are probably the most studied taxa. In three species of earthworms (*Allolobophora chlorotica*, *Apporectodea caliginosa*, and *Lumbricus rubellus*) from a moderately contaminated Dutch floodplain, HNO_3-, $CaCl_2$-extractable and pore water Cd, Cu, Pb and Zn concentrations in soils were non significantly correlated with concentrations in the worm tissues (Van Vliet and Van der Zee, 2005). Total soil concentrations were weakly ($R^2 < 0.10$) correlated with worms for Cd, Cu and Zn but not for Pb. Adding flooding, a frequent environmental stress in those habitats, as a new variable in the models allowed improving R^2 to 0.67 for Cd, 0.10 for Cu and 0.33 for Zn. The relationship remained non significant for Pb. Modeling each species separately, only total soil concentrations were significantly related to metal concentrations in worm tissues (not for all species or metals) with R^2 ranging from 0.14 to 0.80. As in the Dutch case on earthworms, our data showed that Pb concentrations in soils, whatever the extractant used, generally do not correlate with Pb concentrations in snails when all species are considered together. The influence of species is the most important variable explaining snail concentrations. In a study of another Dutch floodplain, Cd and Cu concentrations in *A. caliginosa* and *L. rubellus* have also been found to correlate best with total rather than to pore-water and $CaCl_2$-extractable concentrations (Hobbelen et al., 2006).

In the present work, the use of free metal ion activities in the soil pore water to model metal concentrations in snails gave no better results than chemical extractions. This is in agreement with the results of a study aiming at using *Helix aspersa* snail as quantitative sentinels of total and predicted free Pb concentrations in soils (Beeby and Richmond, 2003). In this work where adult snails were sampled from 23 locations in England and Wales, correlations between free Pb activity calculated using the same equation (Sauvé et al., 1997) and Pb concentrations in snail tissues were low ($R^2 = 0.16$, $p < 0.001$). In a study on *Eisenia fetida* exposed to nine Cd-spiked soils, similar low relationships were found between free Cd activity and Cd accumulation in the worm tissues (Li et al., 2009). However, the introduction of pore water pH as an explanatory variable increased R^2 to 0.45, indicating that Cd uptake by *E. fetida* may be moderated by the presence of other cations in the soil pore water, especially H^+ ions.

In a synthesis of the researches undertaken on Dutch floodplains, van Gestel concluded that bioavailability could not be predicted from available concentrations in pore water or 0.01 M $CaCl_2$ soil extracts (Van Gestel, 2008). Our results on several species of gastropod mollusks reinforce the conclusion that extractable concentrations might not provide predictive models accurate enough to be used in routine risk assessment.

Another key factor in understanding the transfer of metals from the environment to organisms relies on food chain transfers. Indeed, in a study of metal transfers in a soil - plant (*Urtica dioica*) - snail (*Cepaea nemoralis*), relationships between metal concentrations in plants and snails were stronger than between soils and snails (Notten et al., 2005). The authors interpreted this phenomenon as a major contribution of plant materials to the transfers of metals to snails, compared to the contribution of the soil, which was, though, considered significant. In a study aiming at evaluating *H. aspersa* snails as sentinels for mapping pollution, however, correlations between soil and snail Cd concentrations were slightly higher than correlations between *Taraxacum* leaves and snails (Beeby and Richmond, 2002), suggesting a rather equal contribution of soil and plant in the accumulation of metals by the snails. Moreover, the relative contribution of soil and plants in the accumulation of metals by snails has been suggested to vary greatly depending on the metal considered (Scheifler et al., 2006). Therefore, depending on the metal and on the actual diet of the different snail species, relationships between metal concentrations in snail tissues and soil concentrations are likely to drastically vary.

Snails have various diets depending on the considered species (Speiser, 2001) and obviously do not only depend on the soil when considering their exposure and subsequent contamination. Plants of all developmental stages (from seedlings to senescing plants), leaf litter, wood, dead and even alive animals have been recorded as gastropod food (Speiser, 2001). Therefore, when the transfer of metals from the environment to the snail has to be modelled, one should not only consider the soil on which snails are crawling and feeding but also the rest of their diet. However, even in this case, one would obtain metal concentrations in the soils and in various supposed food items, which would not allow modelling the transfers without knowing the proportion of each item is actually ingested.

4.3. Modeling metal concentrations in snail tissues using soil total metal concentrations and properties slightly increases predictions

When data are handled with all species together, models associating total concentrations and soil properties do not explain metal concentrations in snail tissues better than extraction-based ones, the influence of the species remaining the most important variable. Looking at species separately, however, the models using soil properties are generally slightly better than extraction-based. To our knowledge, only few studies investigated the role of soil properties in explaining metal accumulation in wild snail tissues. In the present study, soil pH was found to be inversely related to the accumulation of metals in snails. Soil pH affects the partitioning of metals in the different soil compartments and, therefore, the metal concentration available for exposure from soil solution. Several studies performed on *in situ* contaminated soils with different species of worms found that total metal concentrations in soils was a poor predictor of earthworm metal accumulation due to a number of modifying factors such as pH, organic matter content, and clay particle content (Nahmani et al., 2007). In the present study, metal concentrations in *O. draparnaudi* snails increased with total concentrations and decreased with organic matter values, as also observed on *Lumbricus rubellus* (Corp and Morgan, 1991). This suggests that metal adsorption on organic matter occurred, reducing the bioavailability of metals at high organic matter contents. Clay content has also been found to negatively influence metal availability for animals at high contents (Janssen et al., 1997; Owojori et al., 2010). In our study, clay content was positively correlated with Pb concentrations in the six species considered together and in Cepaea snails, but not in *Oxychilus* individuals. Our results also showed a negative relationship between tissues Cd and Pb concentrations in the six species and in *Cepaea* snails and silt content, but this was not observed in *Oxychilus* snails. Taken together, our results do not show a straightforward influence of soil properties, whose influence was species- and metal-specific.

5. Conclusion

In this study, metal concentrations in snails were poorly explained by total and extractable soil concentrations, whatever the chemical extractant, and by predicted free ion activities in the soil solution. When total metal concentrations and soil characteristics were used as explanatory variables, the explanation of the variations of metal concentrations in snail tissues slightly increased, particularly when data were handled species-specifically, but remained lower than 42%. Biotic parameters like species when a multi-species dataset is studied, or age when it can be determined (i.e. for certain species for which a method exists), were of primary importance in explaining snail tissue concentrations. Taken together, those results suggest that correlations between snail tissues and external (total, or extractable concentrations, predicted free ion activities) are not high enough to be used for predictive purposes. Data coming from laboratory or microcosm studies, particularly those using freshly spiked soils, sometimes show better or even good relationships between invertebrate tissue and external concentrations, and this has lead to consider chemical extractions as a promising tool to predict metal bioavailability. The few field data (mainly on worms and, in the present work, on snails) reinforce the well known feeling that extrapolation from laboratory to field conditions is not straightforward and that both approaches should be combined to understand and to predict bioavailability. Moreover, important insights have to be gained in food web approaches allowing determining food web structure and composition, and hence, a better understanding of the transfer (and subsequent effects) of contaminants in ecosystems.

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Table 1. Descriptive statistics of total concentrations of Cd, Pb and Zn in the 25 cm soil layer (*n* = 48) and in snail soft bodies (*n* = 264), and physico-chemical parameters of soils, from the former "Metaleurop-Nord" smelter (Noyelles-Godault, France). Different letters represent significant differences of metal concentrations between soils and snails according to linear models.

	Cd_{soil}	Pb_{soil}	Zn_{soil}	Cd_{snail}	Pb_{snail}	Zn_{snail}	OM	Clay	Silt	CEC	pH
	$\mu g.g^{-1}$						g.kg^{-1}			cmol^{+}.kg^{-1}	
Minimum	0.7	74.7	87.5	4.3	4.2	164	24	29	90	7.4	4.0
1st quartile	5.3	266	405	24.6	16.9	381	55	146	328	14.0	6.2
Median	10.7	402	672	45.1	33.2	606	78	197	414	19.2	7.8
Mean	11.9^{a}	733^{a}	882^{a}	52.9^{b}	63.0^{b}	836^{a}	107	212	431	18.3	7.1
3rd quartile	14.1	1144	1304	66.7	76.2	1029	150	243	520	21.0	7.9
Maximum	104	2360	3790	258	825	6619	243	391	719	29.2	8.3

Table 2. Partial R² of explanatory variables (when significant) and adjusted-R² of the models (in brackets, when significant) linking snail metal concentrations to soil concentrations (total or extractable) and free metal ion activities in the soil solution (pMetal^{2+}). Best R² are indicated in bold. Negative relationships between metal concentrations in snail tissues and explanatory variables are indicated in between bracket (-). Partial R² of biotic variables (species or age) can be deduced by subtracting values of partial R² of soil concentrations from the R² of the model.

TM	$[TM]_{total}$	$[TM]_{CaCl2}$	$[TM]_{citric\ acid}$	$[TM]_{acetic\ acid}$	$[TM]_a$	$[TM]_b$	$[TM]_d$	$[TM]_r$	pTM^{2+}
6 snail species (n = 264, model: $\log_{10}[TM+1]_{snail}$ ~ species + $\log_{10}[\text{total or extractable TM + 1}]_{soil}$)									
Cd	3.2 (20.2)	5.7 (18.3)	4.6 (20.5)	6.0 (**21.7**)	ns[a] (17.0)	**6.8** (20.2)	ns (14.8)	ns (16.9)	2.3 (-) (15.5)
Pb	ns (6.4)	Ns (5.6)	ns (8.4)	ns (**14.1**)	ns (3.7)	2.4 (-) (11.0)	ns (5.8)	ns (5.9)	**3.5** (-) (8.6)
Zn	2.9 (39.5)	Ns (36.1)	2.0 (-) (38.5)	ns (37.3)	1.1 (36.5)	**3.6** (**42.1**)	1.7 (37.4)	3.1 (38.7)	2.2 (-) (37.8)
Oxychilus draparnaudi (n = 78, model: $\log_{10}[TM+1]_{snail}$ ~ $\log_{10}[\text{total or extractable TM + 1}]_{soil}$)									
Cd	21.9	8.6	23.1	**24.3**	11.5	11.8	5.5	7.3	ns
Pb	7.0	Ns	19.1	23.0	ns	**25.3**	7.4	ns	ns
Zn	13.7	Ns	10.9	6.4	4.4	**25.2**	7.2	7.6	ns
Cepaea sp (n = 131, model: $\log_{10}[TM+1]_{snail}$ ~ age + $\log_{10}[\text{total or extractable TM + 1}]_{soil}$)									
Cd	ns (16.5)	**5.1** (21.7)	ns (16.8)	ns (18.3)	ns (17.8)	4.9 (**21.5**)	ns (20.1)	ns (20.6)	4.3 (-) (20.9)
Pb	ns	Ns	ns	**8.1** (-) (**9.6**)	ns	ns	ns	ns	6.2 (-) (7.6)
Zn	ns	3.2 (3.2)	ns	ns	ns	ns	ns	3.9 (3.9)	**6.4** (-) (**6.4**)

[a]ns: non significant

Table 3. Partial R² of each variable, models and their adjusted-R² (Adj-R²) for relationships linking snail metal concentrations to biotic variables (species and age), external total metal concentrations and soil properties in 6 snail species, in *Oxychilus draparnaudi* and in *Cepaea sp*. The negative or positive relationship between metal concentration in snail tissues and each quantitative explanatory variable is indicated by a sign minus or plus in bracket.

	Species	Age	$[TM]_{total}$	OM	pH	Clay	Silt	CEC	Adj-R^2
6 snail species (*n* = 264)									
Cd	14.9	nc[a]	3.1 (+)	4.6 (-)	ns[b]	ns	2.6 (-)	ns	
	$Log(cd_{int}+1) \sim species + log(cd_{tot}+1) + log(OM+1) + log10(silt+1)$								23.0
Pb	7.0	nc	ns	ns	4.0 (-)	2.5 (+)	1.8 (-)	ns	
	$Log(pb_{int}+1) \sim species + log(pb_{tot}+1) + log10(pH+1) + log10(clay+1) + log(silt+1)$								13.4
Zn	36.8	nc	3.2 (+)	ns	3.0 (-)	ns	ns	ns	
	$Log(zn_{int}+1) \sim species + log(zn_{tot}+1) + log10(pH+1)$								41.6
***Oxychilus draparnaudi* (*n* = 78)**									
Cd	nc	nc	22.9 (+)	8.9 (-)	ns	ns	ns	ns	
	$Log(cd_{int}+1) \sim log(cd_{tot}+1) + log(OM+1)$								30.0
Pb	nc	nc	8.3 (+)	22.4 (-)	ns	ns	ns	ns	
	$Log(pb_{int}+1) \sim log(pb_{tot}+1) + log(OM+1)$								29.0
Zn	nc	nc	15.2 (+)	22.0 (-)	ns	ns	ns	ns	
	$Log(zn_{int}+1) \sim log(zn_{tot}+1) + log(OM+1)$								34.7
***Cepaea sp* (*n* = 131)**									
Cd	nc	18.2	ns	4.5 (-)	3.9 (-)	ns	5.2 (-)	ns	
	$Log(cd_{int}+1) \sim age + log(cd_{tot}+1) + log10(silt+1) + log(pH+1) + log(OM+1)$								29.0
Pb	nc	3.0	ns	4.2 (+)	5.7 (-)	4.2 (+)	6.7 (-)	ns	
	$Log(pb_{int}+1) \sim age + log(pb_{tot}+1) + log10(silt+1) + log(pH+1) + log(OM+1) + log(clay+1)$								20.0
Zn	nc	ns	ns	3.7 (+)	3.5(-)	ns	8.6 (-)	ns	
	$Log(zn_{int}+1) \sim age + log(zn_{tot}+1) + log10(silt+1) + log(OM+1) + log10(pH+1)$								15.1

[a]: not concerned

[b]: not significant

Figure captions

Figure 1. Cd, Pb and Zn concentrations (µg.g^{-1} dw) measured in soft tissues of each snail species sampled in the area of the former "Metaleurop-Nord" smelter (Noyelles-Godault, France). AA: *Arianta arbustorum*, CH: *Cepaea hortensis* (adults), CJ: juvenile of *Cepaea sp.*, CN: *Cepaea nemoralis* (adults), HA: *Helix aspersa* (syn. *Cantareus aspersus*), OD: *Oxychilus draparnaudi*, TS: *Trichia striolata*.

Figure 2. Cd, Pb and Zn biota-to-soil accumulation factors (BSAF) for snails as a function of soil total metal concentrations (µg.g^{-1} dw) for 6 snail species (left) and for *Oxychilus draparnaudi* (OD) and *Cepaea sp.* (Csp) individuals (right) sampled in the area of the former "Metaleurop-Nord" smelter (Noyelles-Godault, France). Lines represent model predictions with associated coefficients (slope and intercept). For the purpose of readability, metal concentration values are given in µg.g^{-1} dw in both x- and y-axis of the figure. The equations exhibit log-transformed values.

Figure 1.

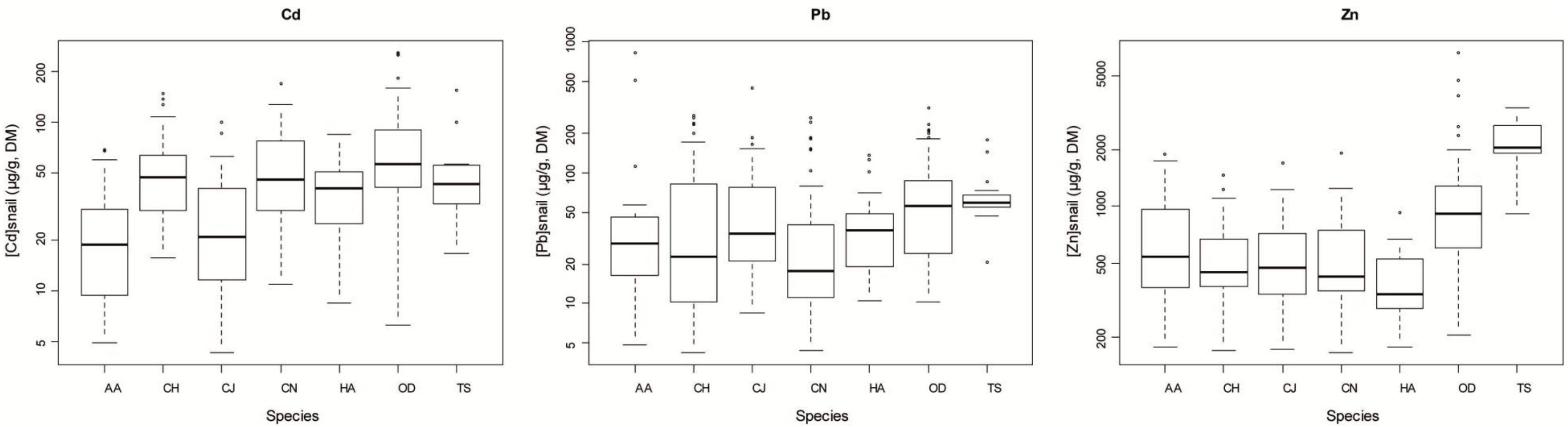
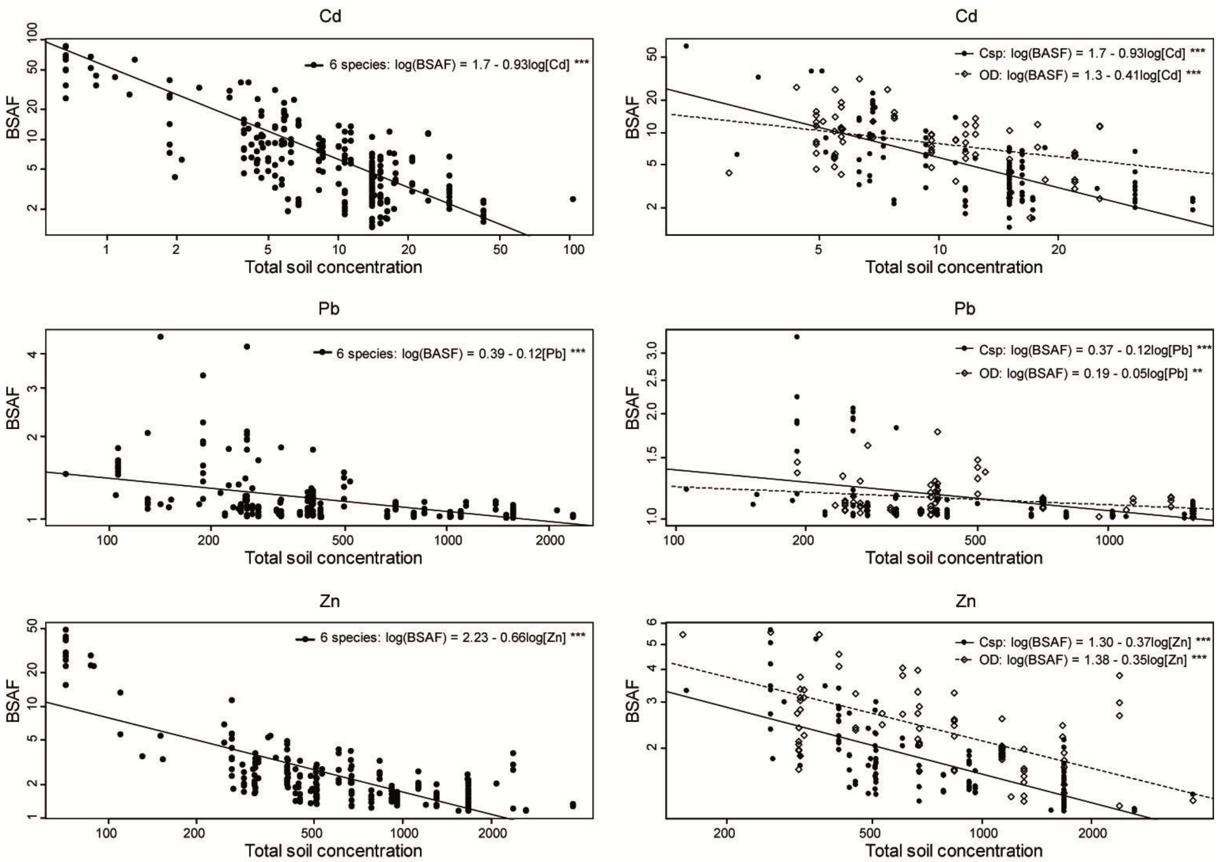

Cd
[Cd]snail (µg/g, DM)
5
10
20
50
100
200
AA CH CJ CN HA OD TS
Species
Pb
[Pb]snail (µg/g, DM)
5
10
20
50
100
200
500
1000
AA CH CJ CN HA OD TS
Species
Zn
[Zn]snail (µg/g, DM)
200
500
1000
2000
5000
AA CH CJ CN HA OD TS
Species

Figure 2.


Cd
6 species: log(BSAF) = 1.7 - 0.93log[Cd] ***
BSAF
Total soil concentration
Cd
Csp: log(BASF) = 1.7 - 0.93log[Cd] ***
OD: log(BASF) = 1.3 - 0.41log[Cd] ***
BSAF
Total soil concentration
Pb
6 species: log(BASF) = 0.39 - 0.12[Pb] ***
BSAF
Total soil concentration
Pb
Csp: log(BSAF) = 0.37 - 0.12log[Pb] ***
OD: log(BSAF) = 0.19 - 0.05log[Pb] **
BSAF
Total soil concentration
Zn
6 species: log(BSAF) = 2.23 - 0.66log[Zn] ***
BSAF
Total soil concentration
Zn
Csp: log(BSAF) = 1.30 - 0.37log[Zn] ***
OD: log(BSAF) = 1.38 - 0.35log[Zn] ***
BSAF
Total soil concentration

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